Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 18

The RAS oncogene story

RAS oncogenes in viruses.

A particularly important family of genes or oncogenes in human cancer, the RAS genes, were first discovered through studies of cancer-causing viruses. Sometime in 1963, Jennifer Harvey, working at the Cancer Research Department of the London Hospital Research Laboratories, was inoculating mice and rats with plasma from a rat that had a virus-induced leukemia. She was routinely transferring the virus from one animal to another, inducing leukemia in each of them. However, on one occasion that year, she noted something unusual that was to open a new window to cancer cause and treatment (Harvey, 1964).

Mice that were inoculated with virus from one of her leukemic rats unexpectedly developed solid tumors in addition to the usual leukemia (which have malignant cells in the blood and lymph nodes instead of in lumps in various tissues). Her leukemia virus was later shown to have picked up (spliced into its genome) a DNA fragment from the rat's own genome. That piece of DNA, which was now part of the genome of the new virus, caused the solid-tumor-type cancer lumps in her mice. Moreover, the new cancer gene was found to be a mutated version of a normal RAS gene. Harvey's name was to become immortalized by the letter H in the newly discovered HRAS oncogene, which was a mutated form of a normal HRAS gene. Harvey's new virus caused cells on the surface of a dish to overgrow to form "foci" (Figure 18.1) in a manner similar to what Weinberg's group later observed in their oncogene studies (Figure 15.3 in Chapter 15). Harvey's virus particles seen in electron microscope images had a remarkable unusual structure resembling spoked wheels (Figure 18.2).

In 1967, W. H. Kirsten and L. A. Mayer detected another virus that produced solid tumors in mice. That virus was later found to have picked up a mutated version of another gene of the RAS family, which became known as KRAS (K for Kirsten) (Kirsten and Mayer, 1967). KRAS became one of the most important cancer genes and was discovered to be mutated in nearly all cases of pancreatic cancer.

These early observations led to enormous research efforts that gave much detailed information about the RAS genes and their cancer-inducing mutations.

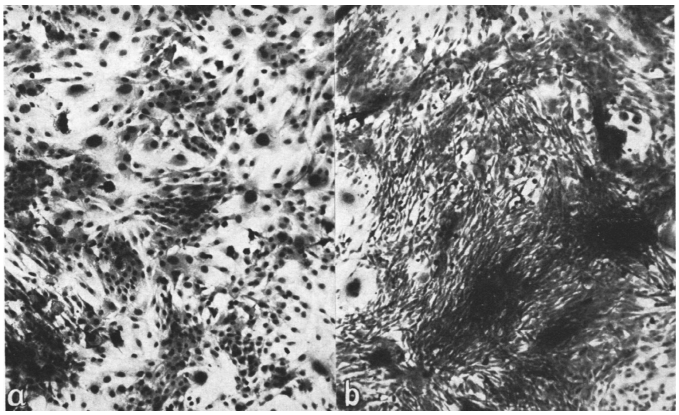

Figure 18.1. Foci of high cell density caused by Harvey's new cancer virus that contained the HRAS gene in its genome. *Left*, normal cells growing on a surface; *right*, foci of excessive cell multiplication caused by the virus (Simons et al., 1967).

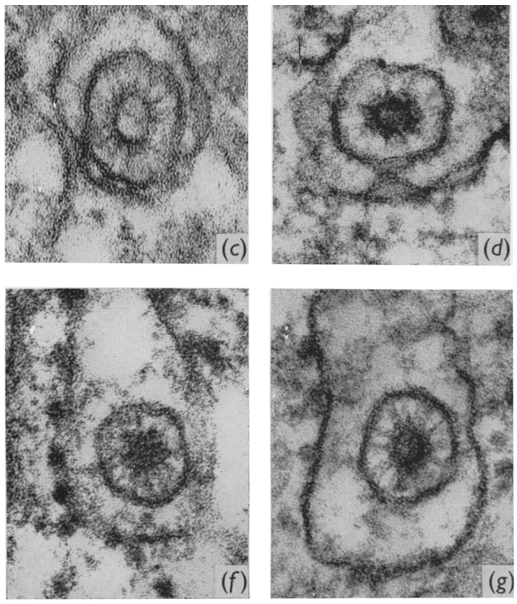

Figure 18.2. Electron microscope images of Harvey's new cancer virus. Each cell sometimes had hundreds of these unusual particles whose structure differed from all previously known viruses. The virus structure resembled spoked wheels within a vesicle membrane that sometimes had ribosomes attached (dark bodies in figure *f* (lower left), indicated that the membrane came from the cell's "rough endoplasmic reticulum" where proteins are made) (De Petris and Harvey, 1969).

What do RAS genes do in cells?

Since a version of the *RAS* gene caused or triggered the development of cancer, researchers were anxious to find out what RAS does in cells. An important observation about the proteins derived from *RAS* genes was reported in 1980 by NIH researchers Mark Willingham, Ira Pastan, Thomas Shih, and Ed Scolnick (Willingham et al., 1980). They found RAS-like proteins at the inner surface of the plasma membrane of cells that had been transformed by Harvey sarcoma virus (Figure 18.3). The result was similar to the observation that epidermal growth factor (EGF) also bound to the cell surface membrane (Figure 17.5 in Chapter 17). The importance of these observations became evident when the role of RAS in the signaling network from receptor tyrosine kinases, such as epidermal growth-factor receptor (EGFR), was worked out -- and when it was discovered that receptor tyrosine kinases, including EGFR, and the RAS proteins were all attached to the cell surface membranes of the cells. As we will see, RAS turned out to be directly in the signaling path from EGFR. (The EGFR story was told in Chapter 17.)

Proteins with structure and function similarities to mammalian RAS were found in a remarkably wide variety of organisms from yeast to worms to insects, which highlighted their central role in the life of many kinds of cells (Sigal et al., 1988) (Lowenstein et al., 1992).

The fact that the cancer-driving RAS genes are mutated versions of the normal RAS genes was reported in 1982 by M. Baracid and his coworkers in the National Cancer Institute (Santos et al., 1982). In 1984, Raymond Sweet and his colleagues at Cold Spring Harbor Laboratory injected the mutated HRAS gene into a variety of cells and found that it increased the proliferation of the cells in cancer-like fashion (Feramisco et al., 1984). The mutated RAS protein (product of a mutated RAS gene) was later found to be a rogue molecule that sent its growth-promoting signal downstream without control and without requiring input from receptor tyrosine kinase.

Overview of RAS in the signaling path from EGFR.

After receiving activating signals from EGFR (or from other receptor tyrosine kinases), RAS transmits the signal to the cell nucleus, telling the machinery therein to activate cell division.

For RAS to receive signals from EGFR, it helps for the two to be located in the same neighborhood. Since EGFR transmits signals from outside to inside the cell, the EGFR molecule is in the cell surface membrane with part of the molecule outside, part within, and part inside the cell (Chapter 17). The location of RAS at the inner surface of the membrane is therefore ideal for efficient interaction with EGFR. It was indeed found that the ability of the RAS protein to bind to the inner surface of the membrane was required for RAS to receive signals from the receptor tyrosine kinases.

However, RAS did not bind directly to EGFR. Instead, there was a protein that connected between the two. This EGFR-to-RAS connector protein came to have a strange name: SOS, standing for "sister of sevenless." The discovery of SOS and the reason for its strange name story came from research on fruit fly eyes, a remarkable story that I will tell next.

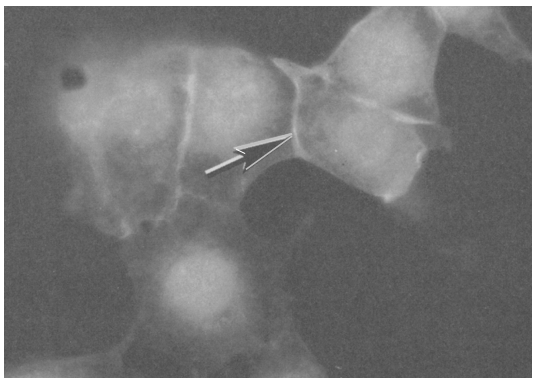

Figure 18.3. An experiment showing that RAS proteins are located at the inner surface of the cell surface membrane (arrow). This experiment was reported in 1980 by NIH scientists Mark Willingham, Ira Pastan, Thomas Shih, and Ed Scolnick (Willingham et al., 1980). They used an antibody that bound to the RAS protein specifically. The antibody's fluorescence under ultraviolet light showed up bright in this image. They also showed that the RAS protein was not on the external surface of the cell: there was no fluorescence when the antibody was applied to intact cells rather than to the fixed cells in the experiment shown here. (The antibody could not penetrate into cells unless the cells were opened up by chemical fixation.)

From viruses and fruit fly eyes to RAS and cancer-driver genes.

Three seemingly unrelated and arcane investigations converged to one of the most important discoveries about cancer: the discovery of the RAS oncogenes, which paved the way for the development of targeted anticancer drugs:

• *A virus unexpectedly produces malignant tumors in mice.*
• *Peculiar mutations in the eyes of fruit flies disclose genes that are similar to previously unidentified human genes.*

• *DNA from human cancer cells transform non-cancerous cells to become cancerous.*

I have already told the first and third of those stories in previous chapters; this section is about the second – an arcane and indeed amazing story about mutations of the eyes of fruit flies.

Who would have imagined that studies of genetic alterations in fruit fly eyes would lead to the discovery of cancer-causing genes and to therapies designed to block those over-active mutated genes in cancers. The story of how that happened is both fascinating and enlightening.

From fruit fly eyes to human RAS genes.

In order to probe the unknown, a key is needed to unlock a door. A key can be found in the most unlikely place -- which, in this case, was memorialized by an unknown (to me) author:

3 blind flies, see how they fly
one was missing the seventh cell
another lost its daughter cell
the third had no mother cell
but it all led to a cancer cure
and never got a golden fleece prize
for 3 blind flies, 3 blind flies.

So, let's have a look at the fruit fly eye and what those missing eye cells were all about. The compound eye of a fruit fly consists of several hundred small eye units, called "ommatidia", each of which has 8 photoreceptor cells arranged in a strict geometric order. Each of those photoreceptor cells was designated by a number, based on its position (Figure 18.4).

A mutation was found in a fly whose photoreceptor cell number 7 was missing in every little eye unit (ommatidium) (Figure 18.4). Geneticists dubbed the mutation *sevenless*, in line with the usual whimsy of those researchers. To have a normal eye, the fly had to have a normal *sevenless* gene. If its sevenless gene was mutated, photoreceptor cell number 7 was missing, and the fly did not see well. To see the drastic effect that a mutation of its *sevenless* gene has on the structure of a fly's eye, have a look at Figure 18.5.

However, geneticists as usual were not content with discovering just one interesting mutation. They observed that the normal development of receptor cell number 7 was defective if there was a mutation in a different gene, which their whimsy dubbed *bride of sevenless*. That name reflected their finding that the protein coded by that gene binds to and is required for the function of the *sevenless* protein.

But the process of finding mutations in fruit fly eye cells did not end there. They found yet another gene whose mutation caused problems with receptor cell number 7. They dubbed that gene *son of sevenless* (SOS). To everyone's astonishment, that SOS gene of the fruit fly had a DNA sequence that resembled a human gene that was implicated in the function of the RAS genes (Raabe, 2000). After much investigation, the human version of the SOS gene was found to fit in the pathway that leads from a variety of receptor tyrosine kinases -- most notably EGFR -- to RAS.

The EGFR story was related in Chapter 17. Figure 18.6 shows the remarkable similarity of the pathways where SOS has a role in transmitting signals from outside the cell to genes in the cell nucleus. The pathways from EGF via SOS and RAS, to RAF, MEK, and ERK were found to be the same in the different species.

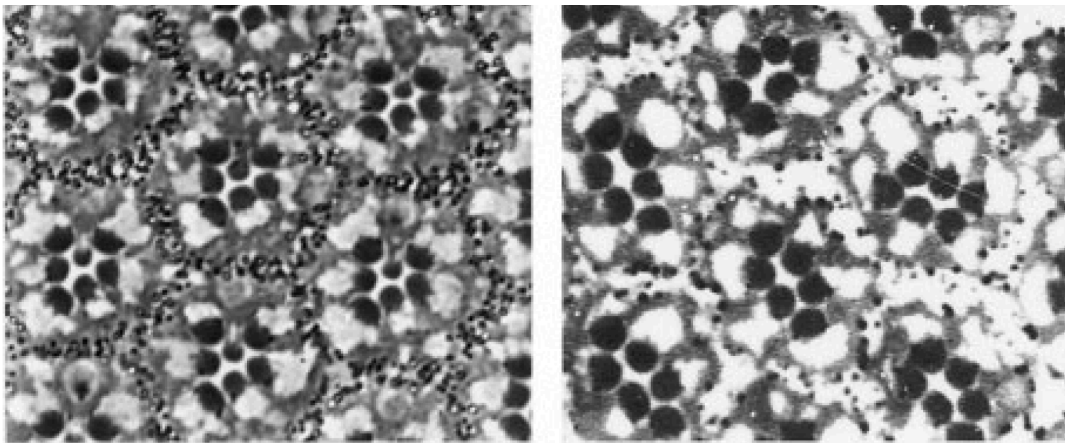

Figure 18.4. Eye units ("omatidia") of a normal fly (left) and a *sevenless* mutant (right). As you can see by counting the dark blobs in each group, the normal fly had 7 photoreceptor cells visible in each omatidium, whereas the mutant had only 6. Photoreceptor cell number 7 was missing in the mutant. (An 8th photoreceptor is not visible in this section and was unaffected by this mutation.) (From (Raabe, 2000).)

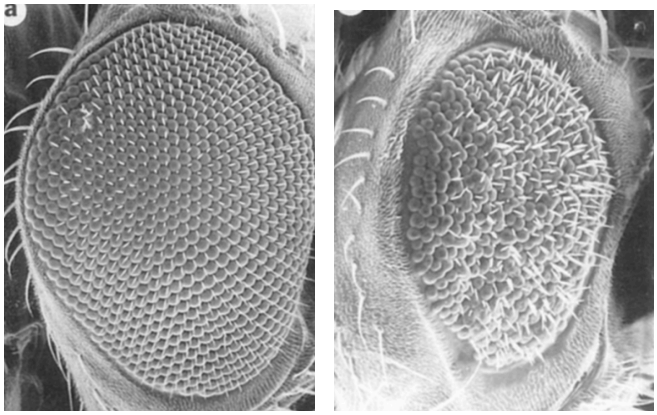

Figure 18.5. How mutation of the SOS gene affects the eye of a fruit fly. *Left,* eye of a normal fruit fly; *right*, eye of a fruit fly that has an SOS mutation (Rogge et al., 1991).

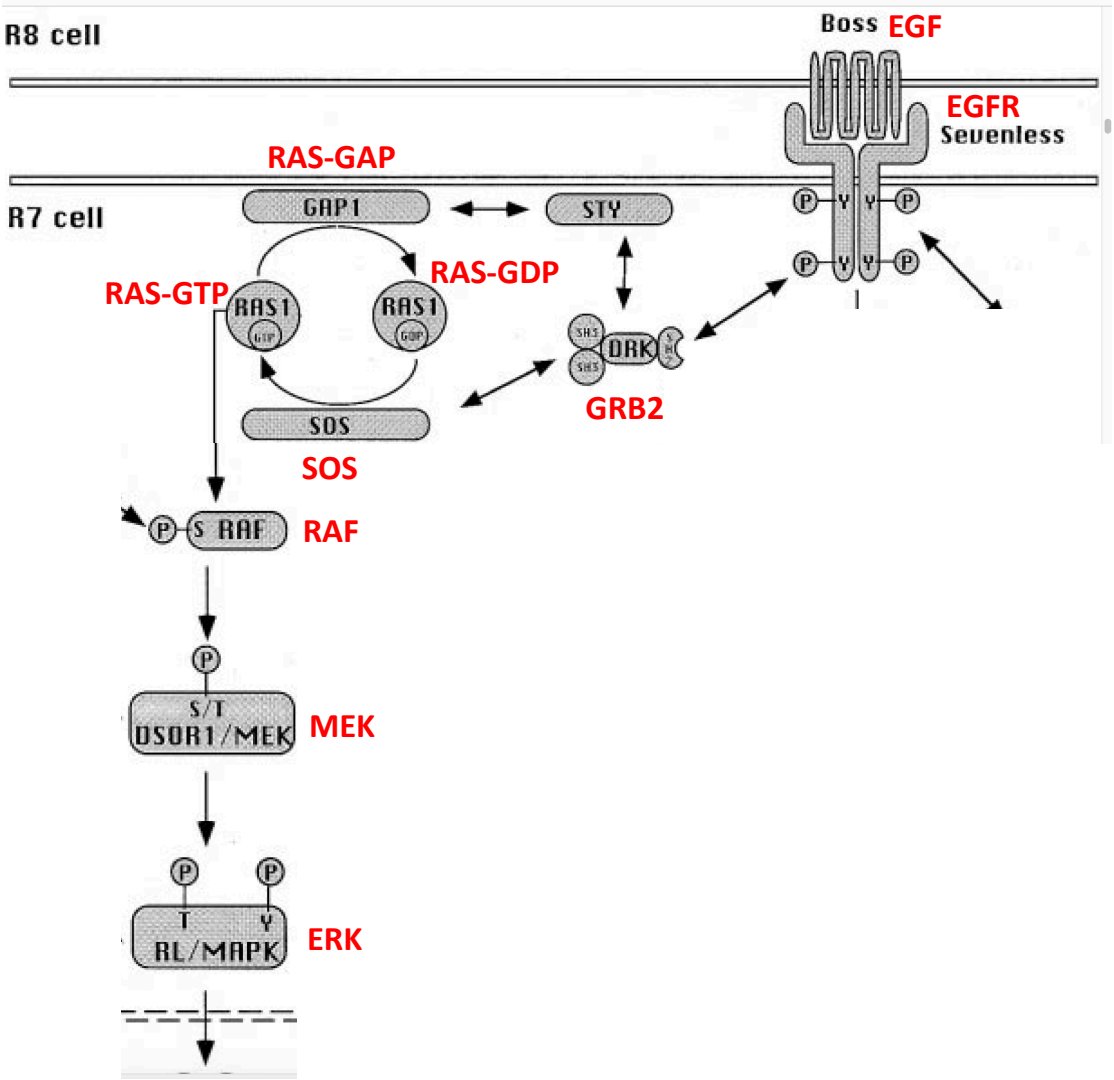

Figure 18.6. The role of SOS in the pathway from EGF to RAS was found to be similar in the fruit fly and in humans, as well as other animals. This diagram shows the pathway in the fruit fly proposed by Thomas Raabe in 2000 (Raabe, 2000). I have added the corresponding human gene names in red. The DNA sequences of the fruit fly genes and the corresponding mammalian genes were similar, although not identical. SOS in both species converts the inactive form of RAS (RAS-GDP) to the active form (RAS-GTP). In humans, the input to the pathway is EGF (epidermal growth factor), which corresponds to the fruit fly's Boss gene ("bride of sevenless"). The output of the pathway from RAS via RAF, MEK, and ERK was also similar in the fruit fly and humans (compare with Figure 18.7). The known functions of the genes at the end of the pathway, however, were different: eye development in the fruit fly versus cell division in humans.

The critical role of RAS genes in transmitting signals from growth factor receptors, such as EGF.

The RAS story expanded enormously as its role in stimulating uncontrolled division of cancer cells gradually emerged from the mist. The strength of the cell division signal from RAS obviously had to be strictly controlled, because excessive cell division could lead to cancer. The control of RAS via positive and negative influences were discovered, and how it all works to control cell division gradually unfolded.

The unravelling of the story began with the fruit fly eye mutation studies described above. The mutated genes were then isolated and their DNA sequences, which revealed the amino acid sequences of the proteins encoded in the genes were determined.

In 1987, Ernst Hafen, Gerald Rubin and their coworkers at the University of California at Berkley located the *sevenless* gene on the fruit fly chromosome (Hafen et al., 1987). They isolated the gene and determined its DNA sequence, from which they surmised that the gene coded for a receptor tyrosine kinase that had the structure of a trans-membrane protein.

In the fruit fly eye, the *sevenless* protein (corresponding to EGFR in humans) on photoreceptor cell R7 bound the *bride of sevenless (Boss)* protein on the adjacent cell R8. In that way, the R8 cell controlled the behavior of the R7 cell. The *sevenless* protein in the R7 cell then signaled, by way of *son of sevenless (SOS),* down the chain to ERK, which entered the cell nucleus to activate genes. If that control was in any way defective due a mutation, the development of the eye was defective and produced abnormal structures, such as shown in Figure 18.5.

Understanding of the fruit fly's signaling from *sevenless* accelerated in the 1990's, particularly in the laboratory of Uptal Banerjee at the University of California in Los Angeles. In 1991, they reported studies of SOS mutants that pointed to SOS being an intermediary between *sevenless* (corresponding to EGFR) and RAS (Rogge et al., 1991). Then in 1992, they sequenced the *SOS* gene and inferred that it served to activate RAS (Bonfini et al., 1992). By 1993, the chain from *sevenless*/EGFR via GRB2 and SOS to RAS had been worked out (Karlovich et al., 1995) (Figures 18.6).

The parts (domains) of those proteins that carried out their respective bindings had also been worked out. The GRB2 protein was found to serve only as a linker between EGFR and SOS. One end of the GRB2 molecule had an 'SH2' domain that was noted to bind to phosphate groups on tyrosine amino acids of proteins. Thus there was a sequence of links from EGFR to GRB2 to SOS to RAS.

When EGFR bound to EGF, a pair of EGFR protein molecules paired up and added phosphate groups to each other's tyrosines at specific places on the proteins (described in

Chapter 17). Those phosphotyrosines then bound the SH2 end of a GRB2 protein. The other end of GRB2 had an 'SH3' domain that bound a particular amino acid arrangement on SOS. That's all that GRB2 was responsible for doing. SOS, on the other hand, not only linked between GRB2 and RAS, but also stimulated the activity of RAS by facilitating the replacement of GDP by GTP on the RAS molecule (Figure 18.6). That chain of proteins then sent signals to the R7 cell urging it to become a photoreceptor in the fruit fly eye.

It is mind-blowing how nearly the same network of protein interactions in a critical control pathway exists in humans as in fruit flies. In the fruit fly, the network controls the development of the eye, whereas in humans it controls cell division. I don't know whether the fruit fly perhaps has another similar network that controls cell division, or whether humans have other networks of this kind that function in the development of the eye or other anatomical structure. Interestingly, the same network arrangement can serve quite different purposes. That fact of nature enabled the extraordinary connection from of fruit fly eyes to human cancer.

How the receptor tyrosine kinase EGFR connects to RAS via SOS and stimulates RAS to signals the cell to divide is shown by the molecular interaction map in Figure 18.7, which builds on the map in Figure 17.6 of Chapter 17. The signal from RAS goes to the cell nucleus by way of a chain of kinase proteins (RAF, MEK, and ERK) that are used by many signaling systems in the cell. (The cell also receives other stimulatory and inhibitory signals that determine whether the signal from RAS is actually executed.)

The interesting way that RAS itself is regulated was shown in Figure 18.6. That regulation is based on the fact that the RAS protein has on it a site that can bind either GTP or GDP (guanosine triphosphate or guanosine diphosphate). When RAS has GTP bound to the site, it is active and sends signals down the pathway to the cell nucleus. When, instead, GDP is bound to the site, RAS is inactive and does not send signals. SOS activates RAS by allowing GDP to be replaced by GTP at the site on the RAS protein. In the opposite direction, a RAS-GAP protein inactivates RAS by stimulating the conversion of the bound GTP to GDP. This balance between activation and inactivation regulates RAS and thereby regulates the strength of the signals sent down the pathway to the cell nucleus.

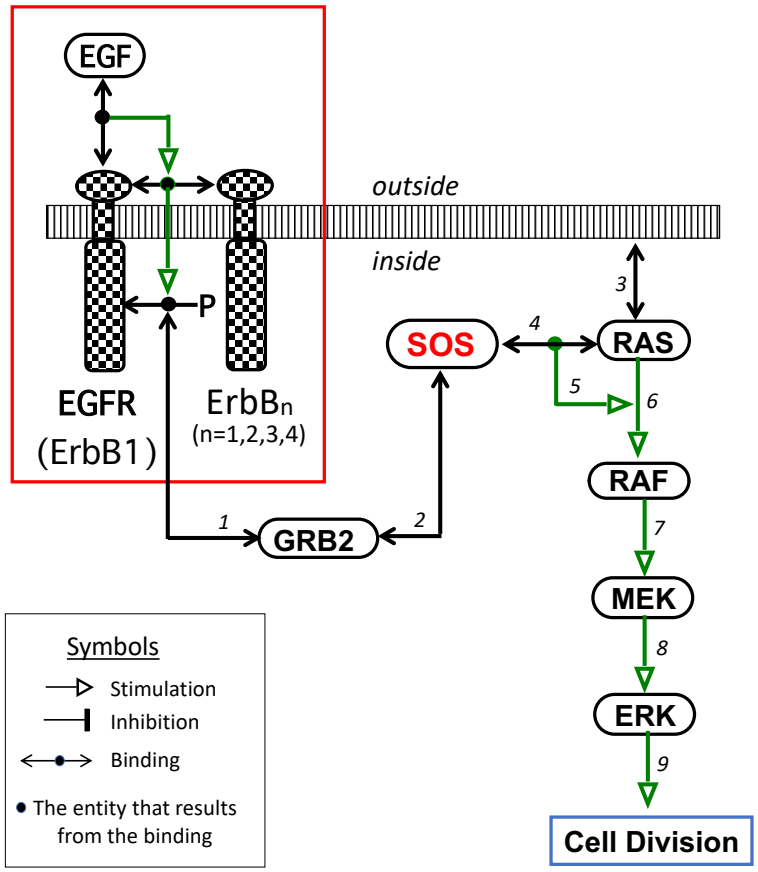

Figure 18.7. How SOS connects growth factor receptors with RAS in the activation of cell division. The epidermal growth factor receptor (EGFR, also known as ErbB1) becomes activated when it binds an epidermal growth factor. EGFR can then bind to another ErbB family member. The two ErbB then phosphorylate each other's intracellular parts (domains). Many sites are phosphorylated, but only one is shown. These events are in a red box, because some details are omitted. The phosphorylated EGFR site then binds the adapter protein, GRB2 *[1],* which then binds SOS *[2]*. That brings SOS to the cell membrane, where both EGFR and RAS *[3]* are located. The combination of SOS and RAS *[4]* then activate RAS *[5]* to send a signal down the RAF-MEK-ERK pathway *[6,7,8]* that simulates cells to enter the cell division cycle *[9]*.

In 1984, an important discovery had connected that story to human cancers. It was found that the RAS genes was often mutated cancer and, furthermore, that the mutation blocked the conversion of the bound GTP to GDP, thereby preventing the inactivation of active RAS (Gibbs et al., 1984). Consequently, the mutated RAS was active all the time and sent excessively strong cell division signals. Thus, when a mutant RAS gene was injected into cells, the cells divided without control, as they do in cancer (Feramisco et al., 1984).

But the question remained: why was the convertion GTP to GDP defective in the mutant RAS protein? The reason turned out to be that this GTPase activity, which is an integral

part of the RAS protein, is normally activated by another protein, RAS-GTPase-activating-protein (RAS-GAP, for short). The defect in the mutant RAS was that it did not respond to RAS-GAP (Trahey and McCormick, 1987) (Vogel et al., 1988). (Like RAS, the RAS-GAP protein binds to the inner surface of the cell surface membrane, thus localizing it to where it can efficiently interact with RAS.)

HRAS and KRAS, together with NRAS, constituted the RAS family of genes having very similar DNA sequences. Taken together, mutations in one of the RAS genes were found in about 10% of all cancers. Of the three RAS genes, KRAS was found to be by far the most frequently mutated in cancer. Remarkably, there was one type of cancer that nearly always had a KRAS mutation: cancer of the pancreas. Other types of cancer that frequently had KRAS mutations were about 45% of colorectal cancers and about 35% of lung adenocarcinomas. HRAS was mutated in about 10% of lung adenocarcinomas. NRAS was mutated in about 15% of melanomas. I don't know (and perhaps no one knows) why RAS mutations are common in only certain types of cancer. In particular, why do pancreatic cancers almost always have a KRAS mutation?

Almost all of the oncogenic mutations of RAS occurred at only 3 sites – the amino acid changes occurred at only 3 position in the chain (Cox et al., 2014). Moreover, the 3 changes all had the same effect: they prevented RAS-GAP from interacting with RAS, thereby keeping the RAS protein continually in its active GTP-bound state. In other words, the GTPase activity of RAS was unable inactivate itself by converting its bound GTP to GDP. Therefore, since RAS was active in its GTP-bound state, but not in its GDP-bound state, the mutated RAS protein remained active all the time and continually sent signals to the cell nucleus to stimulate the cell to divide.

A RAS mutation by itself, however, was insufficient in causing cancer, because other proteins, particularly TP53, could stop the malignancy. A malignant tumor, therefore developed only after developing other molecular defects, particularly an inactivating TP53 mutation. (TP53 is the topic of Chapter …)

Although we understood how these oncogenic mutations induced cells to grow into cancers, how to interfere with that process so as to provide therapy for the 10% of patients whose cancer was driven by a RAS mutation remained a big problem. It was a complex problem, in part because the RAS proteins have several important functions in the cell. Efforts to find a solution were in progress at the time of this writing.

Failure of efforts to find RAS-inhibiting anticancer drugs.

There were several possible ways to suppress the overactivity of mutated RAS. A drug that inhibited any of the many factors that required by RAS to be active might be effective. Despite decades of efforts, however, medicinal chemists had not come up with a clinically approved drug, although it seemed possible that further study might lead to effective drugs (Cox et al., 2014).

Some cancers became addicted to high RAS activity. A drug that inhibited RAS, either directly or in a downstream pathway might then be effective specifically against those cancer cases. Research became directed mainly on KRAS-dependent cancer – where the cancer cells were addicted to high expression of KRAS.

One of the first approaches was to look for drugs that would compete with GTP for binding to the mutant RAS protein. That effort failed, however, because the affinity of RAS for GTP was too high: chemists could not find a drug molecule that could compete with that high affinity.

Blocking the GTPase activity of the RAS protein was of course not a good idea, because it would maintain RAS in its high-activity GTP-bound state. On the other hand, a drug that worked like RAS-GTPase to convert the RAS-bound GTP to GDP would inhibit RAS activity, but attempts to find such a drug also failed.

Another idea was to inhibit the binding of RAS to the cell surface membrane, because that would hinder RAS from receiving signals from EGFR, which was located in the cell surface membrane. Well then, what causes RAS to become bound to the membrane, and could that be inhibited?

To enable RAS binding to the cell surface membrane, the cell has an enzyme that adds a long hydrocarbon chain to the RAS protein. The hydrocarbon chain is lipid-like and tends to merge with the lipid part of membranes, thereby carrying the RAS protein along with it. Inhibitors of that enzyme were therefore considered as drugs that might suppress RAS activity. The problem was that many other essential molecules rely on the same chemistry to carry them to the cell surface, and it was difficult to find a drug specific for the RAS protein. Another problem was that there were different enzymes that linked different kinds of hydrocarbon chains onto RAS and inhibiting any one of those enzymes would still allow a different enzyme to link a similarly effect hydrocarbon chain. Efforts to use this approach, however, were rekindled based on deeper understanding of the relevant molecular complexities (Cox et al., 2015).

In the face of all those difficulties and failures, RAS had become considered to be "undruggable." New technology, however, restored hope that direct targeting of RAS may yet succeed (Cox et al., 2015) (Ryan et al., 2015).

KRAS became the major focus of clinical attention.

The most frequent oncogenes whose over-activity drive human cancers are the closely related members of the RAS family: KRAS, HRAS, and NRAS. Of those, KRAS, was the most important, because it drove the malignancy in about 20% of cancer cases (Downward, 2015), whereas mutations of HRAS or NRAS were less frequent.

Activating mutations of KRAS made it an oncogene, a gene that, together with other factors, initiated cancer. KRAS mutations were extraordinarily common in cancers. Most remarkable was that a KRAS mutation was found in 95% of patients with pancreatic cancer. In addition, such mutations were found in about 40 % of patients with colorectal cancer and in 20% to 25% of patients with adenocarcinoma of the lung.

The mutant KRAS oncogene was discovered in 1983 by Manning Der and Geoffrey Cooper at Harvard Medical School. They discovered an abnormal protein in cancer cells, made by a mutated gene that produced cancer upon transfecting the gene into non-cancer cells. The mutated gene thus was an oncogene -- which they identified as a mutant *KRAS* (Der and Cooper, 1983). Much time and effort was needed, however, to find out what overactive *KRAS* did to make cells cancerous.

In 2009, Jeff Settleman and his colleagues showed that cell lines derived from human lung or pancreas cancers differed in the degree to which they were addicted to KRAS (Singh et al., 2009). They thought that the addiction might make those cancers vulnerable to specific drugs, and they set about investigating whether that approach could lead to drugs that were effective against cancers that were addicted to KRAS. Inhibiting KRAS or its downstream actions might eradicate at least those cancers that were highly KRAS-addicted.

Figure 18.8 shows how they identified cell lines that were highly addicted and that could perhaps be targeted by specific drugs. In order to determine the degree of addiction, they first suppressed the production of KRAS by inserting into the cells a small hairpin RNA (shRNA) that specifically blocked the KRAS messenger-RNA, thereby blocking the production of KRAS protein. Then, they looked to see whether the cells were dying, which would indicate that the cells were addicted and would not be able to survive without KRAS. They did that by measuring the amount of cleaved caspase-3 protein that was produced when KRAS was suppressed. A central feature of cell death by apoptosis was the cleavage of the caspase-3 protein (it is broken into two pieces that then come together in a new configuration to generate an active caspase-3 enzyme that starts the apoptosis process).

Since attempts to develop a KRAS-inhibiting drug had failed, the investigators thought that inhibiting a step downstream from KRAS might work. They therefore set out to investigate the molecular changes occurring when KRAS was artificially suppressed using an shRNA.

Although such RNA's could not become useful drugs, researchers did not give up trying to target RAS. Among many efforts to apply new molecular techniques was the possibility of engineering antibodies that would specifically target mutant KRAS protein inside the cell (Shin et al., 2020).

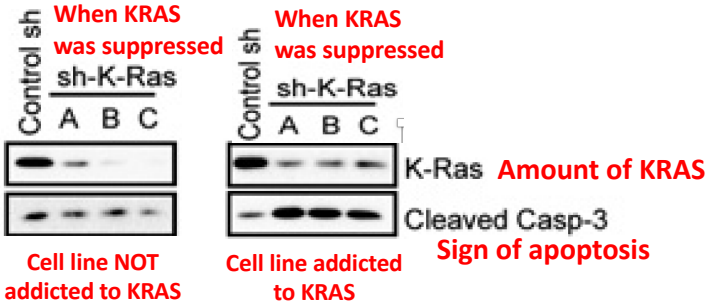

Figure 18.8. An example of two human cancer cell lines that differed in whether addicted to KRAS (Singh et al., 2009). The cell line on the *right* is addicted to KRAS: when the experimenters suppressed KRAS, the cells died by apoptosis. The cell line on the *left* is NOT addicted to KRAS: when the experimenters suppressed KRAS, the cells did not die. In order to tell whether or not the cells were KRAS-addicted, they measured the caspase-3 cleavage product. When the amount of cleaved Casp-3 protein increased in the addicted cell line on the right, it indicated that the cells were dying by apoptosis. There was no increase in Casp-3 cleavage in the non-addicted cell line on the *left.*

Summary

The three *RAS* genes are the most frequently mutated genes that drive human cancer – they are the most frequent oncogenes activated by mutation. Their importance stimulated strong efforts to develop inhibitors of the overactive RAS functions. However, these efforts, extending over more than three decades, were disappointing, giving rise to the opinion that mutant RAS proteins were "undruggable." Armed with new technology and deeper understanding of the complexities of RAS functions, attempts to develop therapy targeted against RAS oncogenes were renewed (Papke and Der, 2017).

Earlier studies – before 2015 -- had revealed that the strength of signals from RAS proteins depends on control of RAS activity. RAS proteins send signals to the cell nucleus to initiate cell division, but this happens only when RAS is in its GTP-bound state. Importantly, the amount of RAS-GTP was tightly controlled, so that cells did not divide too often. That was accomplished by control of RAS cycling between the active GTP-bound state and the inactive GDP-bound state.

This Chapter looked back at how mutations of fruit fly genes led to the discovery of human versions of those genes functioning in an analogous pathway. The fruit fly protein that is altered by the *sevenless* mutation was found to be a receptor tyrosine kinase that corresponded to human EGFR (Simon et al., 1991). The *Son of sevenless* (SOS) mutation was especially revealing, because it disclosed previously unkown genes that turned out to be central to the cause and treatment of many human cancers. Particularly important was the discovery of the *RAS* genes. The relevance of SOS to cancer was shown by finding that it transmits signals from EGFR to RAS. RAS in turn activates RAF, which is the topic of Chapter 19. It is remarkable how that arcane route from fruit fly eye mutations to the RAS

oncogenes, together findings about cancer-causing viruses, led to discovery of many other human oncogenes and their importance in cancer cause and treatment.

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